GENETIC ANALYSIS OF AN ANOMALOUS SEX RATIO CONDITION IN DROSOPHILA AFFINIS

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INTRODUCTION

GENETIC alteration of the normal sex ratio in Drosophila by a disturbance of the meiotic processes provides a means for the investigation of chromosome mechanics, somewhat analogous to the inferential determination of the properties of the wild-type gene by a study of its mutant alleles, and further presents problems of evolutionary importance in those cases where the genetic system responsible is found in natural populations and therefore cannot be dismissed as a laboratory curiosity. Perhaps the most striking example satisfying both these criteria is that of the "sex ratio" gene, which causes males bearing it to produce few or no male offspring. This "sex ratio" gene has been found in a member of the *affinis* group (STURTEVANT in MORGAN, BRIDGES, and STURTEVANT, 1925), in *obscura* (GERSHENSON, 1928), in *athabasca*, *azteca*, *affinis*, and *pseudoobscura* (STURTEVANT and DOBZHANSKY, 1936) and in *melanica* (STURTEVANT in STURTEVANT and NOVITSKI, 1941; SPENCER, unpublished).

GERSHENSON has shown that meiosis of *obscura* females heterozygous or homozygous for this X chromosome gene proceeds normally but that males hemizygous for it produce nearly all X sperm instead of the normal 50 percent. STURTEVANT and DOBZHANSKY have localized the factor (or factors) responsible on the right limb of the X chromosome in *pseudoobscura;* a cytological investigation revealed that the X chromosome of "sex ratio" males undergoes an equational division at each meiotic division, the Y chromosome usually degenerating so that each spermatid generally receives an X chromosome, but no Y.

The anomalous sex ratio to be described here is one which results in the production, by certain *affinis* males, of only male offspring. This phenomenon has been named "male sex ratio" (abbreviated hereafter as MSR), to distinguish it from the opposite condition described above.

MATERIALS

The mutant genes in *affinis* used here include: white (*w*) and miniature (*m*), on the standard sequence of the X chromosome; ascute (*asc*), scarlet (*st*) and hairy (*h*) on the inverted sequence of the X chromosome; jaunty (*jt*) and net (*nt*) on chromosome B; pinklike (*p*) and cinnabar (*cn*) on chromosome C;

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rugose (*rug*) on chromosome E. More detailed information about the nomenclature of the chromosomes may be found in STURTEVANT and NOVITSKI (1941) and NOVITSKI (1946). The Y-autosome translocations made use of in the latter part of the work were selected from the translocations which yielded the original correlation of salivary gland chromosomes and linkage groups in this species.

"SEX RATIO" IN AFFINIS

Since, as will be shown later, MSR is associated genetically with "sex ratio," some of the genetic characteristics of the latter must be considered first. STURTEVANT (1940) has recorded for *affinis* two widely distributed sequences of the X chromosome, "standard" and "inverted." The "sex ratio" effect that has been investigated here is produced by an X chromosome carrying a third sequence. This sequence reduces the crossing over between hairy (*h*) and scarlet (*st*), 62 units apart on the "inverted" sequence, and between white (*w*) and miniature (*m*), 35 units apart on the "standard" sequence, to practically zero. An examination of the salivary gland chromosomes of heterozygotes for the "sex ratio" X chromosome and "standard" show that the former sequence cannot be a simple crossover derivative of the other two.

The ratio of the sexes obtained from "sex ratio" males is quite variable, although the source of all the "sex ratio" X chromosomes used here can be traced to one such X chromosome found in a series of cultures manifesting the MSR effect. In one set of 8 cultures, an average of 170 ♀♀ to .6♂♂ per culture was obtained; in another set of 18, the average ratio per culture was 97 ♀♀ to 46♂♂. These data suggest the presence of one or more modifying factors on the other chromosomes, but an analysis of these has not been attempted. The possibility of mistaking "sex ratio" cultures for normal ones and vice versa has been reduced by using whenever possible only "standard" and "inverted" sequences marked with recessive mutants, their respective wild-type alleles automatically marking the "sex ratio" sequence.

DISCOVERY OF THE MSR PHENOMENON

During the course of the translocation studies above mentioned three cultures, 1-17, 1-21 and 1-24, yielded only male or almost only male offspring. The nature of the matings which showed this effect may be described as *cn rug* ♀♀ × F_1 (*cn rug* ♀♀ × Woods Hole X-rayed ♂♂) ♂♂. Two transfer cultures of 1-17 produced a total of 12 ♀♀ and 208 ♂♂, culture 1-21 produced 10 ♂♂ and culture 1-54 produced 25 ♂♂. This phenomenon was later observed by PROFESSOR A. H. STURTEVANT in the F_2 from a pair mating of F_1 individuals from a cross of a *p* ♀ by a *cn* ♂. Only 1 ♀ but 64 ♂♂ constituted the entire progeny of this bottle.

The original discovery of MSR in crosses involving irradiated chromosomes had suggested that the causal factor was an induced mutation or a chromosomal aberration. The later appearance of this condition in cultures derived only from wild strains negates that possibility. The fact that all four crosses showing this effect involve stocks carrying *cn* is strongly suggestive of the presence in the *cn* stock of the genes responsible.

THE GENETIC COMPOSITION OF MSR MALES

The preliminary crosses designed to isolate the aberrant genetic system responsible for MSR were rather ineffective. Several pertinent points were, however, established. The appearance of MSR in cultures in which the female parents were of the compositions *w*, *h st*, *asc st*, *h st/w*, *h st/+*, *w/+*, and *jt net* showed that the genetic composition of the male alone was the determining factor. Inbreeding experiments indicated that the genetic basis of MSR was not simple, and that "sex ratio" was in some way associated with it. The rather high sterility of MSR males and the unisexual inclination of the cultures from both MSR and "sex ratio" males made inbreeding difficult. Outcrossing individuals from the strains known to contain, to some degree, the MSR complex to mutant strains for the purpose of marking the chromosomes generally led to the loss of the MSR effect, as tested by subsequent inbreeding. In only one case was the outcrossing followed by a recovery of the MSR effect; the strain extracted has been the basis of all later work.

The single female appearing in the F_2 of the mating of a *p* ♀ × a *cn* ♂ was crossed with a *w* male. Tests by mating to *w* females of 16 of the male offspring from this cross showed clear evidences of the presence of a "sex ratio" X chromosome (to be denoted as *sr* hereafter) in five of the males. Females of the constitution *sr/w* from the culture showing the most extreme sex ratio (101 ♀♀ : 1 ♂) were mated to *w m* males in order to obtain a stock carrying the *sr* X chromosome in a balanced condition. After one generation of inbreeding (*sr/w m* ♀♀ × *w* ♂♂) males carrying *sr* were tested and were found, in a few cases, to produce only male offspring; the majority of the fertile cultures showed the typical "sex ratio" phenomenon. A large series of matings of the type *w/sr* ♀♀ × *w m* ♂♂ was set up, with a subsequent selection of those in which at least some of the males carrying the *sr* X chromosome produced only male offspring. The strain finally derived, of which all fertile males having the *sr* X chromosome also showed the MSR effect, regardless of the genotype of its mate, was maintained by selection of *sr/w m* ♀♀ and *w m* ♂♂ as the parents for each generation.

An obvious conclusion is that the *sr* X chromosome is a necessary prerequisite for the MSR effect of a male. Never has a male with "standard" sequence (marked by *w*) produced only male offspring; certain cultures involving *h st*, in which all the *sr* males from a *h st/sr* female were MSR and all *h st* males were not, indicate that the "inverted" sequence is also not involved in MSR.

Several types of matings indicate the nature of the difference between "sex ratio" males and MSR males. If a female of the constitution *w m/sr* is outcrossed to certain unrelated strains, as *asc st* or *jt net*, the F_1 *sr* males invariably show the typical "sex ratio" effect. MSR males can be found among the *sr* males of the F_2. When the male parent comes from the same inbred strain as the female parent, all the *sr* males show MSR.

The above results can be explained by the assumption that an autosomal recessive gene (*a*) present in the homozygous state in *sr* males (*sr*, *a/a*) converts it from a "sex ratio" male into a MSR male. Assuming that this factor

exists, there is no evidence that it has any effect in the heterozygous state.

In order to demonstrate the existence of the *a* gene, an attempt was made to localize it on a particular chromosome. Pair matings of *w m/sr* females, of the inbred MSR strain and so with the hypothetical constitution *w m/sr, a/a*, were mated to males carrying *jt net* (chromosome B), to males carrying *cn* (chromosome C) and to males carrying *rug* (chromosome E). Stocks of the constitution *w jt net*, *w cn* and *w rug* were derived from this mating to eliminate the unmarked X chromosomes from the autosomal mutant stocks. The factor *a* might be present in these stocks (1) if selection for the mutant genes did not eliminate it by virtue of their location on different chromosomes or by virtue of an appreciable amount of crossing over between it and the mutant gene if they were on the same chromosome, and (2) if the *a* factor was present in the original autosomal mutant individuals mated to *w m/sr, a/a* females. This latter possibility was most serious for the cross involving *cn*, because of the evidence from earlier crosses which had indicated the presence of the *a* factor in the *cn* stock. Tests of F_1 *sr* males, which should reveal whether the particular mutant individuals used carried the *a* factor, either in the homozygous or heterozygous state by the appearance of the MSR effect either in all or in half the males, gave negative results.

Males from the multiple mutant stocks were then mated to *w m/sr, a/a* females and F_1 *sr* males were tested for the presence of the homozygous *a* factor in the way described above as a test for the presence of the *a* gene in the autosomal mutant stocks.

The results of these tests were quite striking: the *w rug* strain was homozygous for the *a* gene, the *w cn* strain was heterogeneous for it, and the *w jt net* strain did not carry it at all. A strain of the composition *w m/sr, a, rug* was immediately obtained; a comparable strain homozygous for *cn* instead of *rug* was obtained within two generations. The attempt to obtain the comparable strain carrying *jt net* failed completely; this suggests that *jt net*, and *a* were on homologous chromosomes and that selection for *jt net* automatically eliminated *a*.

Corroboratory evidence that *a* is located on chromosome B has been obtained in another way. Segregation of a Y-autosome translocation in a male heterozygous for it is such that the chromosome which has exchanged fragments with the Y chromosome will generally be present in all males. Consequently, repeated backcrossing of such males to females carrying marker genes on the chromosome corresponding to that involved in the translocation is ineffective; the males will always be heterozygous for those chromosomes involved.

With the series of Y-autosome translocations available in *affinis*, such a test is relatively simple. Males carrying the translocations involving chromosome B (TY-4A), involving chromosomes B and C simultaneously (TY-3B), involving B and E (TY-4E), and involving chromosome E (TY-2A, TY-2B) were mated individually to females of the composition *w m/sr, a*. F_1 *w m* males, heterozygous for the translocation and for *a*, were backcrossed to *w m/sr, a* females. If the gene *a* is borne by the chromosome corresponding to the one in-

volved in the translocation, all the *sr* males from this cross must be heterozygous for *a* and so will behave as "sex ratio" males. If the gene *a* segregates independently of the translocated chromosomes, half the *sr* males of the backcross generation will be heterozygous for *a* and behave as "sex ratio" males, the other half will be homozygous for *a* and consequently may be expected either to be MSR or to show a characteristic sterility. Another possibility is that the translocated chromosomes of any given aberration may themselves carry *a* rather than its wild type allele, since MSR was first discovered in that set of cultures from which the translocations were derived. In this case such backcrossing should lead to *sr* males of which all would be expected to show the MSR effect instead of "sex ratio." These two expectations, males all of one kind or of two kinds have been represented in table 1 by the numbers 1 and 2, respectively, for the translocaions used, when *a* is assumed to be located on each of the three long autosomes.

TABLE 1

The numbers of the kinds of males expected from back crosses to w m/sr, a females of males carrying certain translocations, considering individually the various possibilities for the location of "a" on the three long autosomal chromosomes.

TRANSLOCATION	POSITION OF *a*		
	IF ON CHROMOSOME B	IF ON CHROMOSOME C	IF ON CHROMOSOME E
Y-2A	2	2	1
Y-2B	2	2	1
Y-3B	1	1	2
Y-4A	1	2	2
Y-4E	1	2	1

From these crosses, males of two different kinds of behavior were actually produced. In the order of the translocations as given in table 1, the different types of male per series were 2, 2, 1, 1, 1 or exactly the distribution expected if *a* is located on element B as previous observations indicated.

The two distinct types of males were not "sex ratio" and MSR, however, but "sex ratio" and normal. All of the cultures (17) of the TY-3A series were clearly "sex ratio," the average number of females and males per culture being 77 and 4.5 respectively. The 50 cultures of the TY-4E series showed an average of 70 females and 4 males. The 49 cultures of TY-4A, on the other hand, gave an average of 95 females and 85 males. In the TY-2A series nine cultures were "sex ratio"; four were normal; three were doubtful and three were sterile. In the TY-2B series, 22 were "sex ratio"; 18, normal and 13 sterile.

The conclusion to be drawn from the above results is that the presence of a Y translocation in a male of the composition *sr a* inhibits the male-producing mechanism so that normal offspring are produced. It follows also that the Y-4A translocation must have the *a* gene on the translocated chromosome B.

Because of this unexpected effect of the Y chromosome, the possibility that

a certain type of Y is involved in the MSR complex must also be considered. With the data available, this cannot be excluded conclusively. However, outcrosses of the inbred MSR strain were generally made using females, which should ordinarily not carry the Y of the MSR strain. The MSR males of the F_2 have the Y of the parental males which, in these crosses, come from a large variety of strains not showing MSR. The specific Y might, of course, be introduced by the parental female if she were XXY. However, since the two X's of such a female have inversion differences (standard/*sr*), one might reasonably anticipate a small percentage of secondary non-disjunction which was in fact not observed although the crosses for the most part involved markers which would have revealed it.

THE CHARACTERISTICS OF MSR MALES AND THEIR PROGENY

During the course of this work, a total of 95 fertile cultures involving MSR males has yielded an average of 25.5 individuals per culture. Females appeared in ten of the cultures with an average of 3.2 per culture that produced females. This gives a total sex ratio of 76.8 males per female.

The male offspring are morphologically normal. They carry an X chromosome of the mother (*w/h st* ♀♀ × MSR ♂♂→*w* ♂♂ and *h st* ♂♂). A cytological analysis of spermatogonial metaphases of three progeny of MSR males crossed to females from the same inbred line revealed that the F_1 males either may (one case) or may not (two cases) receive the Y chromosome of the father. This observation is in agreement with the fact that both *w* and *h st* males produced from such crosses tend to be sterile more often than fertile (in one series, for instance, 22 fertile males carrying "standard" or "inverted" sequences, to 38 sterile ones). The ventral receptacles of the females from four such sterile cultures were examined and proved to be devoid of sperm.

The occasional female offspring of a MSR male have one X chromosome from each parent (*w* ♀ × MSR ♂→mostly *w* ♂♂ rare *w/sr* ♀♀). Whether or not they also may have the Y of the male parent has not been established. Such females are normal, with regard both to appearance and to fertility.

When females mated to MSR males are dissected and the ventral receptacles examined, copious quantities of sperm are generally (seven cases out of eight) found therein, although such females have usually produced no or very few offspring. The sperm, if capable of fertilization, must have a lethal effect upon the zygote. The recessive nature of the factor *a* indicates that this potential lethal effect originates during spermatogenesis rather than at the time of fertilization. This time of origin is also suggested by the fact that the MSR complex has as its basis the *sr* X chromosome, which exerts its influence during spermatogenesis also.

GEOGRAPHICAL DISTRIBUTION OF THE *sr* X CHROMOSOME AND OF *a*

The *sr* X chromosome has been found in strains from Woods Hole, Massachusetts; Coffeyville, Kansas; Gatlinburg, Tennessee (STURTEVANT, 1940); it has also been found in the stock of *p* (origin, Austin, Texas). The factor *a* can be traced in various crosses to the stocks of *p*, *cn*, *h st* (origin ambiguous, in-

cluding strains from both Gatlinburg, Tennessee and Woods Hole, Massachusetts), and also in wild type strains from Plano, Texas and from Woods Hole, Massachusetts.

From these points of distribution it may be surmised that both the *sr* X chromosome and the *a* factor are present to some extent over the entire range of *affinis*. However, additional data on the geographical distribution of these factors do not confirm this surmise. The data were obtained when the loss during the war of all stocks homozygous for either or both of these elements made necessary an attempt to resynthesize the MSR complex. For this purpose there were analyzed the Plano and Woods Hole strains and twenty other wild type strains recently collected in the Southeast. The *sr* X chromosome used was derived from a Woods Hole stock. By the use of an X-ray induced dominant *Minute* mutation on chromosome B, it was possible, in three generations, to make individual chromosomes B homozygous in the presence of the *sr* X chromosome. Because of sterility only 29 chromosomes out of a total of over 130 started were completely tested. These included: one chromosome B from each of seven strains from Alabama, six strains from Georgia, and five strains from Florida; four samples from the Woods Hole stock and five from the Plano stock. In none of these tests was there any evidence of the MSR effect, although typical "sex ratio" appeared in the strains from Georgia and Mississippi. The absence of the *a* factor from the older Plano and Woods Hole stocks was not surprising, for it was anticipated that the lowered fertility characteristic of the homozygotes would lead to the eventual elimination of *a* from the heterogeneous laboratory stocks. The absence in the newer strains, however, must mean that either the gene is not as frequent in wild populations as previous observations indicate, or that its distribution does not include the southeastern area.

DISCUSSION

The behavior of populations of those species of Drosophila having factors altering the sex ratio is difficult to understand. From the evidence obtained from laboratory cultures, the *sr* X chromosome should automatically increase in frequency each generation and it can be inferred that wild populations in which this type of chromosome is found should soon become almost completely homozygous for it (GERSHENSON, 1928). Such a population, the few males in which would produce all or almost all female offspring, would remain in constant danger of extinction. STURTEVANT (unpublished) has suggested that the division of Drosophila species into subpopulations may be of significance in this respect. If the *sr* X chromosome is present in some of these subpopulations, it may frequently lead to their complete elimination. Consequently the frequency of the *sr* X chromosome in the total population may remain at a low level. In this connection it may be pointed out that while the 1:1 ratio of sexes characteristic of so many bisexual species is, fundamentally, a condition imposed by the meiotic mechanism and fulfills the basic requirements for survival it does not necessarily represent the optimum ratio, which may be quite different from one species to another, as well as within a species in different

ecologies. The low male:female ratio expected of subpopulations carrying *sr* may impart up to a point a selective advantage.

Since half the autosomal genes in a population come from each sex, the genetic contribution per individual to the succeeding generation is greater for the less prevalent sex. If, as is ordinarily true, the gene frequencies are the same for both sexes, this consideration has little effect on the composition of the following generation other than the greater statistical variation of gene frequencies in smaller populations. However, any genotype which tends to be more frequent in one sex than the other when the sex ratio is unequal undergoes a change in frequency from one generation to the next even though there is no obvious selective advantage to that genotype. For instance, a new allele present in a single male which mates with nine females constitutes only five percent of the alleles at that locus in that population of ten individuals but would ordinarily reach a frequency of .25 in the following generation. This 500 percent increase is obviously not caused by the greater selective advantage of the allele but by the greater reproductive efficiency of the male by virtue of the inequitable numbers of the two sexes.

Using the above argument, we may consider the situation in a population carrying the *sr* X chromosome. If in that population any new genotypes were to arise by mutation or recombination which allowed males carrying the *sr* X chromosome to produce some male offspring, the additional male offspring would carry the genotype not with its original frequency, but with one considerable higher. The frequency in the total of males would therefore increase, and their greater reproductive efficiency would lead to a considerable increase in the frequency of that genotype in the following generation. The increase would, fortunately for the population, be greater the greater the deficiency of males.

The customary criterion for the identification of the *sr* X chromosome in a male is the absence of male offspring. This unfortunately excludes identification of suppressors, unless they act rather weakly. Nevertheless, evidence that suppressors, probably recessives, actually exist has been found in *affinis* (above) and *pseudoobscura* (STURTEVANT and DOBZHANSKY, 1936). The deleterious effects of a very low male:female ratio might be circumvented by the conversion of "sex ratio" into "male sex ratio" but the efficacy of the factor *a* investigated here in accomplishing this in nature is open to question since the laboratory tests indicate that just those males which would serve as the agents of a frequency increase are largely sterile. However it is noteworthy that, besides the male-producing gene found in *D. melanogaster* by GOWEN and NELSON (1942), which may or may not have evolutionary significance, a rather typical "male sex ratio" effect has been found in *D. pseudoobscura* independently by K. J. MAMPELL (unpublished) and B. WALLACE (unpublished), in the latter case involving the *sr* X chromosome.

SUMMARY

The production of only male offspring by certain males of *Drosophila affinis* has as its basis a genetic constitution involving the "sex ratio" X chromosome,

which ordinarily causes males carrying it to produce only daughters, and a recessive gene on chromosome B. In the presence of Y-autosome translocations the system yields a normal sex ratio. It is suggested that the factors yielding such aberrant sex ratios have an evolutionary significance in allowing certain populations to approach an optimum sex ratio with the possibility of an immediate check should the optimum be exceeded and the survival of the population endangered.

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